Increased Axon Number in the Anterior Commissure of Mice Lacking a Corpus Callosum

By: D. J. Livy, P. M. Schalomon, M. Roy, M. C. Zacharias, J. Pimenta, R. Lent, and Douglas Wahlsten

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Abstract:

Relatively few behavioral deficits are apparent in subjects with hereditary absence of the corpus callosum (CC). The anterior commissure (AC) has been suggested to provide an extracallosal route for the transfer of interhemispheric information in subjects with this congenital defect. Anterior commissure size, axon number, axon diameter, and neuronal distribution were compared between normal mice and those with complete CC absence. No difference in midsagittal AC area was found between normals and acallosals, nor were differences found in the numbers or diameters of myelinated axons. However, axon counts indicated an 17%increase or about 70,000 more unmyelinated axons in the AC of acallosal mice, and the mean diameter of unmyelinated axons was slightly less than in normal mice (0.24 vs 0.26 μm). This decrease in axon diameter enabled more axons to pass through the AC without increasing its midsagittal area. The topographical distribution of neurons sending axons through the AC, assessed with lipophilic dyes, was qualitatively similar for almost all the known regions of origin of the anterior commissure in normal and acallosal mice. There was a pronounced deficit of AC cells in the anterior piriform cortex of BALB/c mice, but this occurred whether or not the mouse suffered absent CC. Although the increase in AC axon number is far smaller than the number of CC axons that fail to reach the opposite hemisphere, the higher number of axons present in the AC of acallosal mice may contribute to the functional compensation for the loss of the CC.

Article:

INTRODUCTION

Congenital absence of the corpus callosum (CC) arises from a defect of the substrates that guide callosal axons across the telencephalic midline (30, 48). Despite the absence of this large interhemispheric communication route, very few behavioral deficits are obvious either in mice (2, 28) or in humans (23, 33, 45). This sparing of functions contrasts with effects of surgical transection of the CC in adult humans, often used in the control of epileptic seizure activity, resulting in a variety of perceptual, motor, and cognitive deficits (4, 14, 24, 49). Although some recovery from the effects of transection in adult humans is apparent (22, 42), this recovery does not lead to the same level of performance achieved in callosal agenesis. Persson (40) found no deficit in the transfer of information between hemispheres despite complete CC agenesis and therefore proposed that extracallosal pathways were used. It has been suggested that the anterior commissure (AC) acts as this route in humans (36). Hypertrophy of the AC in acallosal patients has been reported in several different studies (8, 50). Rauch and Jinkins (41) reported an increase in AC area in 10% of patients with callosal agenesis but a decrease in another 10% of patients.

Whereas the role of the AC in human CC agenesis remains uncertain, it is apparent that putative CC axons from dorsal cortex of mice with hereditary callosal agenesis are usually not rerouted through the AC (38, 39, 54), although Lent (25) found a small number of neocortical axons in the AC of hamsters with surgically induced CC agenesis. Monotremes and marsupials have no corpus callosum and instead the anterior commissure is relatively larger than in eutherians, becoming the main interhemispheric route (6, 9). However, this situation is

not in any way an abnormality, and the topographical distribution of neurons with interhemispheric projections via the AC of marsupials is remarkably similar to the pattern that occurs via the CC in rodents.

If plasticity of anterior commissure development compensates to some extent for the congenital absence of the corpus callosum, it is possible that this could occur via an increase in axon number that also increases AC size or a larger number of smaller diameter axons that does not alter AC cross-sectional area. In either case, higher axon number could represent a greater density of AC projection neurons within the normal sites of origin or a topographical expansion of the zones of origin, most likely at the periphery of normal sites where there is overlap between fields of AC and CC neurons (31). In this study we compare AC size, number of axons, axon diameters, and the distribution of commissural neurons between acallosal and normal mice using morphometric measurement, electron microscopy, and dye tracing techniques.

The inbred mouse strains BALB/c and 129 show incomplete penetrance (55), such that only about 20% of the mice lack a CC. Littermate pairs of BALB/c mice, one with a normal CC and the other with a small or absent CC, have no significant difference in the cross-sectional area of the AC as seen with a lipid stain (54, 55). It remains possible that expansion of the AC would occur in other inbred strains or perhaps hybrid mice that are better able to adjust their brain structures to accidents of ontogeny. We now report data from a total of 1305 mice involving more than eight different inbred and hybrid genetic backgrounds that were examined in the course of ongoing studies of the mode of inheritance of absent CC (44, 56, 57). These data prove beyond reasonable doubt that AC size is not increased in the slightest amount when the CC is absent.

Gross morphometric size of a commissure is not a perfect indicator of axon number, and axon number can differ substantially between groups or at different ages, even though cross-sectional areas are similar (18). Accordingly, we present here axon counts of the AC obtained using electron microscopy, taking care to compare acallosal mice with controls appropriate for their genetic backgrounds. The counts reveal many more unmyelinated axons in mice lacking a CC.

The normal rodent AC shows a somewhat different developmental pattern from that of the CC. Callosal projections are typically diffuse in the early postnatal period and then gradually are pruned to yield the patchy topographical pattern seen in the adult (7). On the other hand, the AC forms prior to the CC in the embryo (48) and its transcortical projections are confined to specific sites from the outset (27). For this reason, it would not be surprising to find that the surplus AC axons in CC agenesis represent a greater density at existing sites rather than occupancy of novel sites. Tracing AC axons to their cells of origin with lipophilic dye confirms this supposition.

METHODS

Experiment 1: AC Morphometry

Mice were reared in the laboratory according to standard conditions described previously (30, 56). The inbred strains 129/ReJ and I/LnJ were obtained from the Jackson Laboratories, Bar Harbor, Maine, whereas BALB/cWah 1 mice were from the colony of D.W. at the University of Alberta. Twenty-three recombinant inbred lines derived from 129/ReJ and BALB/cWah1 had been inbred by full-sib mating for at least seven generations when these data were compiled (57). Brains were also studied from an F_2 hybrid cross of BALB/cWah1 and 129/ReJ (56) as well as three crosses of BALB/ cWah 1 × 129/ReJ F_1 hybrids with I/LnJ (44).

Brains were fixed with 4% buffered neutral paraformaldehyde either by intracardiac perfusion or immersion and then trimmed to a standard configuration (56) and weighed after at least 1 week in fixative. Most brains were bisected at the midsagittal plane and then one hemisphere was stained *en bloc* with the gold chloride method of Schmued (46), although a few were sectioned at 30 µm, mounted on glass slides, and stained. The resulting image revealed all the major forebrain commissures and allowed rapid measures of cross-sectional areas and lengths with a video image analysis program (JAVA from Jandel Scientific). Care was taken to distinguish the

CC proper from adjacent fiber tracts such as the longitudinal striae of Lancisius, the dorsal commissure of the fornix, and the fornix superior (56).

Experiment 2: Electron Microscopy

Animals. The F_2 hybrid offspring from hybrid $129CF_1$ parents (129/J females × BALB/cWah1 males) and two recombinant inbred (RI) lines obtained from $129CF_2$ pairings were bred and raised at the University of Alberta. The recombinant inbred lines were in the sixth generation of inbreeding (57). RI-1 has complete absence of the CC similar to that seen in the strain I/LnJ (28, 55), whereas RI-22 shows consistently normal callosal structure. We also examined a few normal F_2 offspring bred in Alberta from hybrid $B6D2F_1$/J parents (C57BL/6J females × DBA/2J males) obtained from the Jackson Laboratories.

Histology and microscopy. Complete details of tissue processing are provided elsewhere (29). Briefly, all mice were perfused intracardially with about 10 mL of 10 m*M* phosphate-buffered saline (pH 7.6) followed by 80–100 mL of 3% para formaldehyde, 1.5% glutaraldehyde, and 0.02% $CaCl_2$ in 0.1 *M* cacodylate buffer (pH 7.2). Brains were weighed and then 25- to 50-µm slices were cut sagittally with a DSK-1500E microslicer. Adjacent tissue was stained with gold chloride (46) to reveal gross commissure sizes, whereas several sections at the midsagittal plane were further fixed over-night at 4°C in 4% glutaraldehyde in 0.1 *M* cacodylate buffer. Sections were postfixed with 1% OsO_4, 1.5% $K_3Fe(CN)_6$, and 0.02% $CaCl_2$ in 0. 1 *M* cacodylate buffer for 3 h, stained in 2% uranyl acetate (aq) in 0.1 *M* sodium acetate for 45 min and then dehydrated, infiltrated with a graded series of Epon 810, and cured for 48 h at 60°C.

Tissue blocks were trimmed to expose the anterior commissure and 1-µm-thick sections were cut using glass knives on a Reichert–Jung Ultracut E ultramicrotome and stained with toluidine blue O. Detailed tracings of the AC outline, distinguishing between the anterior (AAC) and posterior (PAC) limbs, were made from these sections at 400X. Thin sections (90 nm gold) were then cut using a DuPont diamond knife, stained for 2 h with 4% uranyl acetate (aq) and 1 min with Sato's lead citrate (aq), and viewed and photographed on a Philips 400 TEM at a magnification of 1800×. Fifteen photos were taken of the anterior part of the AC (AAC) and 10 photos were taken from the posterior part (PAC), each at the approximate middle of the grid pore, and the selected grid pores were evenly spaced across the AC, while avoiding the AC edges, the junction between AAC and PAC, and tissue damage.

Axon counts and measurements. Axon counts and diameters were determined from 20 × 25-cm photo-graphic prints from the approximate middle of each negative to yield a final enlargement factor of 16,500×, which gave an unmyelinated axon of 0.25 µm diameter an image 4 mm wide in the print. A 15 × 20-cm counting region was outlined in the middle of each print, and the number of myelinated and unmyelinated axons within the outline was counted. Of the axons touching the border of the template, only those touching the top or left border were included in the count. Axon diameters were measured separately for myelinated and unmyelinated axons using two different 15 × 20-cm templates with fine wire producing a grid with 12 intersection points. A coin toss was used to determine which template was to be used for myelinated and unmyelinated axons, and this template was placed over the outline used earlier for counting. The diameter of the axon closest to each grid intersection was measured. The diameter of nonspherical cross-sections was considered to be the largest distance across the shorter of the axon's outline dimensions. All counts and measures were done without knowledge of the mouse's identity or CC status. Axon counts were extrapolated to the appropriate total area in the Epon section to estimate total axon number.

Statistical analysis. Data were analyzed using multiple regression with effect coding to test three orthogonal (tolerance ≥ 0.97) main effects: (a) corpus callosum presence ($129CF_2$ acallosal and RI-1 mice vs $129CF_2$ normal and RI-22 mice), (b) genetic background ($129CF_2$ vs recombinant inbred), and (c) normal versus abnormal strains ($B6D2F_2$ hybrids vs the four groups derived from BALB and 129). The strain effects (b and c) were included as statistical controls but could not be interpreted unambiguously because the groups differed in age (Table 2). Because of the relatively small sample size, it was not feasible to evaluate interactions with

adequate power (53), although the data were inspected for signs of strain-specific effects. Only effects significant at substantially less than $\alpha = .05$ were considered worthy of attention.

Experiment 3:Distribution of AC Commissural Neurons

The distribution of AC neurons during postnatal development was described using 11 normal B6D2F_2 or F_3 animals, 10 BALB/cWah1 mice, and 11 C129F_3 mice (the F_3 hybrid between BALB/cWah1 and 129/ReJ). Good quality staining was obtained from at least 1 animal of each group at postnatal ages P1,P5, and P14, and additional normal hybrids were observed at P0 and P20. Each pup was perfused intracardially using 4% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4). The brains were then extracted from the skull and bisected at the midsagittal plane. Each hemisphere was transilluminated with a fiberoptic light positioned in order to differentiate the AC by its refringency from the surrounding tissue. With the aid of a stereomicroscope, a small crystal of DiI (1,1'-dioctadecyl-3,3,3',3'- tetramethylindocarbocyanine perchlorate, Molecular Probes) was inserted in the middle of the AC using the tip of a fine dissecting pin (27). After dye insertion, the hemispheres were placed in fresh fixative and stored in the dark at room temperature for 4 to 16 weeks, depending on the age of the pup. The hemispheres were then embedded in a mixture of gelatin and albumin hardened with a few drops of 25% glutaraldehyde. Coronal sections were cut at 100 µm using a vibratome and then counterstained using DAPI (4',6-diamidino-2- phenylindole dihydrochloride, Molecular Probes), mounted on gelatinized glass slides out of 1% buffered paraformaldehyde, coverslipped, and sealed with nail polish. All sections were analyzed using a Zeiss Axioplan fluorescence microscope equipped with filters for rhodamine for observing DiI. The motorized stage of the microscope was connected to a microcomputer equipped with software for 2D reconstruction (Morfo v1.2, Programa Avanc¸ado de Neurociincias, UFRJ). Selected sections were charted or photographed for documentation.

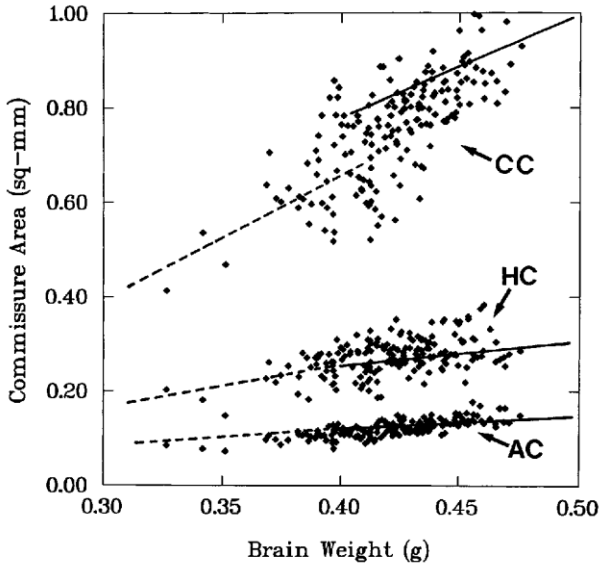

FIG. 1. Cross-sectional areas of the CC, HC, and AC versus weight of the whole brain. Solid lines represent the regression of adult (>50 days old) commissure area on brain weight, whereas dashed lines show the regression for mice between 21 and 42 days old. Equations used to obtain expected commissure areas from brain weight (BR) were as follows. Adult mice > 50 days old: $E(CC) = -0.1 + 2.2BR$; $E(HC) = 0.1 + 0.4BR$; $E(AC) = -0.02 + 0.33BR$. Weanling mice less than 50 days old: $E(CC) = -0.42 + 2.7BR$; $E(HC) = -0.1 + 0.9BR$; $E(AC) = -0.05 + 0.41BR$.

TABLE 1

Linear Relation Between AC Index of Abnormality (Y) and CC + HC Index of Abnormality (X)

Group	Sample size	Intercept ($Y\|X = 0$)	Slope (b_1)	r^2	P
B6D2F_1 and F_2 (normal)	182	0.39	0.62	0.26	<0.001
BALB/cWah 1	161	0.88	0.07	0.02	0.05
129/ReJ	82	1.01	−0.05	0.01	0.31
I/LnJ	50	0.96	0.02	0.00	0.86
F_2 from 129 × BALB	139	0.88	0.03	0.02	0.14
RI lines from 129 × BALB	688	0.91	0.02	0.01	0.04
I/LnJ crosses	185	1.01	0.04	0.01	0.18

Note: The regression lines in the form $Y = b_0 + b_1X$ are shown as solid lines in Fig. 2 for the six genetically abnormal groups. The r^2 value is the proportion of variance in the AC index accounted for by its linear relation with the CC + HC index, and P is the probability of a Type I error when there is actually no correlation between the two variables in the population.

RESULTS

Experiment 1: AC Morphometry

In normal hybrid mice that never have defects of forebrain commissures, the CC, hippocampal commissure (HC), and AC are all larger in larger brains, as shown in Fig. 1 for the B6D2F_1 and F_2 hybrid crosses of the inbred strains C57BL/6J and DBA/2J. Consequently, we have used these normal animals to construct an *index of abnormality* for commissure size in relation to brain weight (57). First, the regression equation of commissure size versus brain weight was estimated for the normal hybrids, which requires slightly different

equations for weanling and adult mice (see Fig. 1). For a mouse of any other strain, the equation then specifies what commissure size is expected on the basis of its brain weight if it were also normal like B6D2 hybrid animals. For example, if the brain weighs 500 mg, the expected CC size is E(CC) = -0.1 + 2.2(0.5 g) = 1.00 mm^2. The index of abnormality is the *ratio* of the actual commissure area to the expected area. If the CC is totally absent the index will be 0, whereas it should be close to 1.0 for a normal brain. In the B6D2 sample the range is from about 0.7 to 1.3; hence a mouse from another strain with an index less than about 0.65 would be considered statistically abnormal.

Absence of the corpus callosum occurs in the embryo and is preceded by retarded formation of the hippocampal commissure (30). If HC formation is sufficiently delayed, many putative HC axons continue growing ipsilaterally and the adult HC at midplane is remarkably small (HC index < 0.65). This condition occurs only when the CC is totally absent. It appears that CC absence and deficiency of the HC are part of the same process of retarded fusion of the telencephalic midline, deficient HC being a more extreme form of the defect. By averaging the CC index and the HC index, the combined CC + HC index yields a value near 0.5 for normal HC but no CC, whereas a value less than 0.3 indicates totally absent CC and abnormally small HC. The question of whether the AC is enlarged when the CC or even the HC is reduced can thus be answered by comparing the AC index with the CC + HC index. In the normal B6D2 hybrid mice there is a positive and significant correlation between these two indices (Table 1), meaning that some animals tend to have larger commissures independently of their brain sizes. On the other hand, if an enlarged AC compensates to some extent for absent CC or perhaps even reduced HC, the correlation should be negative.

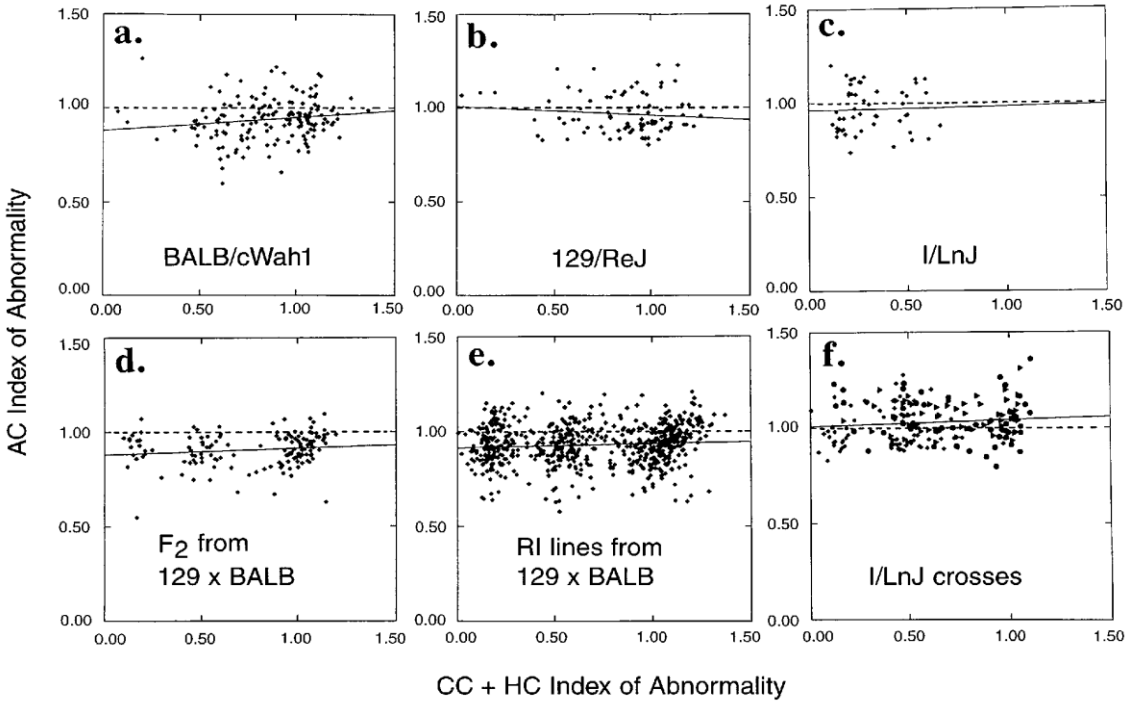

FIG. 2. Index of abnormality for the anterior commissure (AC) versus index of abnormality for the combined corpus callosum (CC) and hippocampal commissure (HC) for six genetically different groups. For the CC + HC index a value near 0.5 indicates that the CC is totally absent, whereas a value less than 0.3 means the CC is absent and the HC is abnormally small. The solid line shows the regression of the AC index on the CC + HC index (see also Table 1), whereas the dashed line at AC index = 1.0 represents the line expected if there is no relation whatsoever between defects of the CC or HC and the size of the AC.

Figure 2 shows the relation between the AC index and CC + HC index for six groups of mice that suffer absent CC and reduced HC with various frequencies. The linear relations between the AC and CC + HC indices are summarized in Table 1. Three of these groups are genetically uniform inbred strains where the defect shows various degrees of incomplete penetrance. In the BALB/cWah1 (Fig.2a)and 129/ReJ (Fig. 2b) strains, about 15 to 20% of mice have no CC but only 4% show small HC, whereas in I/LnJ (Fig. 2c) 100% lack a CC and about 60% have reduced HC. The F1 hybrid of BALB/cWah 1 and 129/ReJ is almost entirely normal, which suggests that these strains differ at two or more genetic loci (55, 57). However, their F_2 hybrid (Fig. 2d) has abundant genetic variation and expresses a high frequency of absent CC as well as about 15% reduced HC (56).

Recombinant inbred lines (Fig. 2e) formed from this F_2 hybrid show an even higher frequency of absent CC and reduced HC because the defects are recessive and inbreeding increases the expression of recessive characteristics. Indeed, three new recombinant inbred lines have emerged that express 100% absent CC and almost always suffer reduced HC, a phenotype that is more severe than the inbred strain I/LnJ (57). Finally, Fig. 2f presents data from three crosses of a BALB/cWah1 × 129/ReJ hybrid with the I/LnJ strain (44), where the crosses differ in the proportion of the I/LnJ alleles at each relevant locus. As shown previously (28), the higher the proportion of I/LnJ alleles, the more common is absence of the CC.

The linear regression equations plotted in Fig. 2 and described in Table 1 prove conclusively that the AC is not enlarged when the CC is absent or even when the HC itself is reduced. This conclusion is based on 1305 mice from several genetic backgrounds. In no group does the linear relation between AC index and CC + HC index reach an adequate level of statistical significance, and the proportion of variance in AC index ''accounted for'' by the CC + HC index never exceeds 2%. When the CC is absent and the HC is reduced, there is no noteworthy increase in AC size.

Experiment 2: Electron Microscopy

Useful data were obtained for 15 mice, 6 of which had no CC (Table 2). Differentiation between the AAC and PAC was facilitated by increased myelination in the AAC (51; see Figs. 3a and 3b). The external border of the AC was defined by an abrupt decrease in the presence of axons or by the presence of longitudinal axons of the dorsal stria (5). In both normal and acallosal brains, distinct and very dense fields of unmyelinated axons were seen in certain areas around the perimeter of the AC (see Fig. 3c), and they often overlapped the border between AAC and PAC. These axons were probably part of the commissural division of the stria terminalis which crosses midline in close association with the AC (5), and they were not included in the area measurements or axon counts.

In a preliminary analysis, no significant effects of sex were found ($P > 0.05$). Although the small sample sizes were inadequate to exclude a small effect of sex, males and females were pooled for further analysis. Normal and acallosal mice in the F2 and RI groups did not differ significantly in brain weight or midsagittal AC ,AAC, and PAC areas, but HC area was substantially reduced in the acallosals ($P < 0.0001$).

TABLE 2

Measures of Individual Mice Used for Electron Microscopy

Strain	Sex	Age (days)	Body weight (g)	Brain weight (g)	CC area (mm^2)	HC area (mm^2)	AC area (mm^2)
$B6D2F_2$	F	99	24.8	0.401	0.684	0.160	0.092
$B6D2F_2$	F	99	24.5	0.427	0.909	0.160	0.109
$B6D2F_2$	F	99	24.9	0.413	0.619	0.134	0.086
$129CF_2$	F	68	21.7	0.442	0.922	0.226	0.105
$129CF_2$	F	67	21.0	0.503	1.079	0.293	0.134
$129CF_2$	M	67	29.3	0.436	0.757	0.318	0.097
$129CF_2$	M	69	24.3	0.448	0.000	0.181	0.096
$129CF_2$	M	68	20.3	0.398	0.000	0.061	0.086
RI-22	F	110	27.9	0.474	0.856	0.337	0.119
RI-22	M	110	31.6	0.460	1.003	0.309	0.124
RI-22	M	110	31.7	0.430	0.850	0.287	0.112
RI-1	F	109	31.1	0.465	0.000	0.046	0.124
RI-1	F	109	25.0	0.468	0.000	0.046	0.121
RI-1	M	109	26.0	0.436	0.000	0.137	0.121
RI-1	M	109	26.3	0.433	0.000	0.078	0.120

Reliability of axon counts. Two series of axon recounts on new photographic prints were made blind by the original counter (P.M.S.) to determine the reliability of the counting protocol. In 15 prints, recounts were performed using the same area outline to determine if the criteria used to differentiate between axon types and extracellular material were reliable. In another 15 prints, the area outline from which the counts were made was moved to a different location of the photograph. Count versus recount correlations for myelinated and unmyelinated axons all exceeded $r = 0.97$ in both sets of 15 recounts.

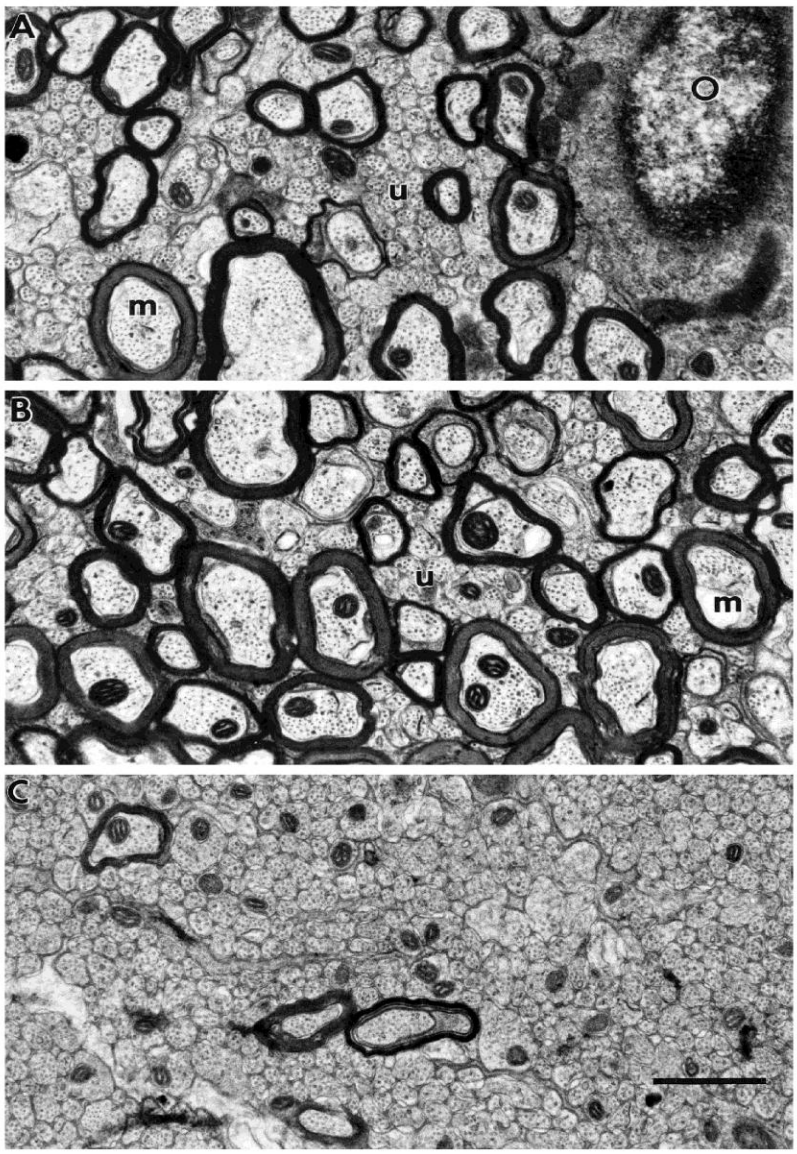

FIG.3. ***(a) PAC area of a B6D2F$_2$ mouse showing fields of myelinated (m) and unmyelinated (u) axons, as well as an oligodendroglial cell (o). (b) AAC area from the same mouse showing the higher proportion of myelinated axons. The fields of unmyelinated axons seen around the perimeter of the AC are shown in (c). Note the consistent size and shape of these axons; they are likely part of the commissural division of the stria terminalis (bar, 1 μm).***

Axonnumbers. The total number of AC axons in the acallosal animals was 17% higher than in the normal animals (534,000 vs 457,000; $P = 0.0004$), but this difference was specific for the unmyelinated axons, which were 21% more abundant (426,000 vs 354,000; $P = 0.00005$, see Table 3). Because the anterior (AAC) and posterior (PAC) limbs of the AC originate from different sites (16), it is possible that axon number may be influenced by CC absence in only one of these areas. The differences found in the AAC were similar to those found in the total AC. Acallosal animals had 26% more unmyelinated axons in the AAC (195,000 vs 155,000; $P = 0.0003$). In the PAC, there was no significant difference in myelinated axon number between the acallosals and normals, but acallosal mice had 16% more unmyelinated axons (233,000 vs 200,000; $P = 0.008$), an effect that was pronounced in the RI strains but not the F_2 mice.

Axondensities. Axon density, calculated as the number of axons in a 1 000-μm^2 zone of gold chloride-stained commissure, adjusts for any possible effect of commissure area on axon number. Acallosal mice had a 33% higher density of unmyelinated axons than normal mice ($P = 0.005$), whereas no differences in myelinated axon

density were apparent (Table 3). The effect of CC absence on unmyelinated axon density was clearly present in the AAC but less so in the PAC.

Axon diameters. There could be more axons in the AC of acallosal animals without a corresponding increase in AC size if their diameter is reduced. Unmyelinated axon mean diameters (Table 3) were indeed slightly lower in the acallosal animals in the entire AC, involving both the AAC ($P = 0.02$) and the PAC ($P = 0.04$), whereas no noteworthy differences were found in the diameters of myelinated axons. A relatively high proportion of axons was of smaller diameter and, as reported by Sturrock (51), no axons less than 0.24 μm in diameter were myelinated; however, about 4% of the unmyelinated axons were larger than 0.6 μm, the largest being 1.5 μm.

Experiment 3: Distribution of AC Commissural Neurons

The distribution of labeled AC neurons in $B6D2F_2$ and F_3 animals was compatible with previous descriptions in other rodents (16, 27). In short, labeled neurons were found in the anterior olfactory nucleus, anterior and posterior piriform cortex, olfactory tubercle, perirhinal cortex, agranular insular area and temporal cortex, and amygdaloid nuclei. Apart from a small sector of temporal cortex (13), no labeled cells were seen in neocortical regions. The distribution of AC-labeled neurons in BALB/cWah1 and $C129/F_3$ mice was similar to that of $B6D2F_2$ and F_3, except for the anterior piriform cortex. In 16 of 21 of these animals, there was a marked decrease in the number of commissural cells within the anterior piriform cortex, and 5 animals showed total absence of labeled neurons (Fig. 4). Surprisingly, the commissural hypoplasia in the anterior piriform cortex was not related to the callosal deficit; rather, it appeared in almost all animals of BALB/cWah1 and $C129/F_3$ genotypes. The commissural zones of origin other than the anterior piriform cortex presented com-parable numbers of labeled cells in the three strains, indicating that the results cannot be ascribed to technical uncertainties such as insufficiency of diffusion time and incomplete labeling.

TABLE 3

Mean values for the total AC of ®ve groups of mice and statistical evaluation of effects of absent CC

			Myelinated axons			Unmyelinated axons		
Genotype	CC size	AC area	Axon no.	Axon density (no./1000 μm²)	Axon diameter (μm)	Axon No.	Axon density (no./1000 μm²)	Axon diameter (μm)
$B6D2F_2$	Normal	0.096	95,846	1018.0	0.698	393,331	4162.1	0.195
$129CF_2$	Normal	0.112	93,915	864.0	0.786	382,557	3491.6	0.257
$129CF_2$	Absent	0.091	102,746	1135.0	0.799	447,401	4940.8	0.243
RI-22	Normal	0.118	113,767	963.1	0.798	324,521	2748.2	0.271
RI-1	Absent	0.122	113,329	932.8	0.795	405,205	3336.7	0.234
SD		0.012	9,885	168.5	0.025	21,304	510.3	0.019
CC effect size (*d*)		0.66	0.39	0.66	0.19	3.18	1.86	1.32
P (1 tail)		0.18	0.28	0.18	0.38	0.00005	0.0046	0.016

Note: Diameter of myelinated axons includes the myelin sheath. Standard deviation (SD) for each measure is that of the residual from the multiple regression model with three effects and 11 degrees of freedom. Effect size (*d*) is the unbiased estimate of the difference between means of the two normal and the two acallosal groups, divided by the pooled standard deviation within groups. An effect size exceeding 1.0 is considered large. The probability of Type I error (*P*) is based on the one-tailed *t* test of the CC effect in the regression model.

DISCUSSION

These results clearly show a large increase in the number of axons in the anterior commissure of acallosal mice. Although this increase was specific for unmyelinated axons, it was found in both the anterior and the posterior halves of the AC. The increase in the number of unmyelinated axons was accompanied by a decrease in the diameter of these axons, resulting in no net increase in AC size. Although there was a strain-specific deficit of neurons projecting from the anterior piriform cortex through the AC, this was unrelated to absence of the CC. The distribution of AC cells of origin was remarkably similar in acallosal mice and their within-strain normal controls.

The substantial increase seen in unmyelinated AC axon number is far less than the number of interhemispheric axons lost due to the absence of the corpus callosum. Extrapolating the number of callosal axons in the rat (12 million in 2.563 mm^2, Ref. 10) to the mouse provides an estimate of 3.5 million in $B6D2F_2$ mice and 4.3 million in $129CF_2$ mice. A rough estimate of callosal axon number based on electron microscopy is 7.1 million in a 42-day-old female $B6D2F_2$ mouse (unpublished observation by D.J.L.). The increase in unmyelinated axons through the AC may help to compensate for this loss of callosal axons by improving the efficiency of information transfer between the hemispheres in the acallosal animals. However, the large net loss in commissural axon numbers, particularly myelinated axons, may explain the increased time required for

information transfer between hemispheres (34) and the decreased coupling of cortical activity in acallosal mice (37).

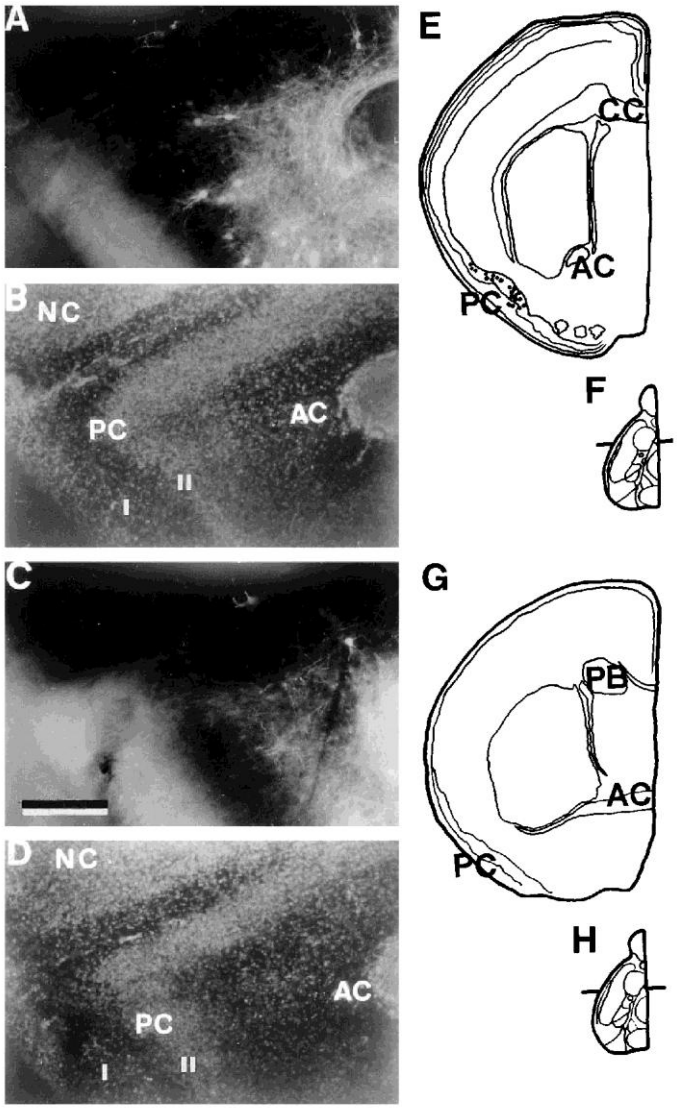

FIG. 4. Coronal sections through the brain of a P5 B6D2F$_3$ mouse at a level caudal to the anterior olfactory nucleus, showing AC neurons in the piriform cortex, backlabeled with DiI (A). The same section, illuminated to reveal DAPI stain, is shown in B. C and D are pictures of a section at a similar level through the brain of a C129F$_3$ mouse lacking the corpus callosum, illuminated respectively for DiI (C) and DAPI (D). In both animals, a normal terminal ®eld of anterogradely labeled ®bers can be discerned in layer I (A and C). Calibration bar, 200 μm. Diagram E shows the distribution of commissural neurons in the pyriform cortex of a normal B6D2F$_3$ mouse, compared with G that shows the absence of labeled neurons in a genetically abnormal F$_3$ mouse (derived from a BALB/cWah1 × 129/ReJ cross). The coronal levels charted in diagrams E and G are shown in F and H.

The origin of extra AC axons remains uncertain. No evidence was obtained by DiI labeling of rerouting by putative callosal axons through the anterior commissure, supporting previous findings with other tract tracing methods (38, 39, 54). All labeled axons were traced back to normal sites of AC origin and there appeared to be qualitatively normal numbers of labeled cells except in the anterior piriform cortex. This plasticity in the number of AC axons, associated with normal and even subnormal numbers of commissural cells, may result from transient branching of fibers within the AC, as shown for the hamster CC (17). Alternatively, the results could be explained by a decrease in the amount of axon elimination after an initial period of overabundant proliferation. Axon elimination following a period of rapid axon production during early development has been shown in the corpus callosum of the rat (10), cat (1), and monkey (20). Similarly, a developmental elimination

of AC axons has been reported in monkeys (21) and opossums (3). However, in both rats (11)and hamsters (27)this elimination of AC axons was not apparent, which may have been due to a simultaneous production and elimination of axons resulting in no net change in axonal number. Alternatively, the AC in primates and marsupials is mainly of neocortical origin, while in rodents the AC is mainly of paleocortical origin, and therefore the occurrence of axon elimination may be a developmental strategy that is more typically employed by the neocortex (26, 27). In fact, a small number of transient, bicommissural neurons has been detected by double labeling in developing hamsters (12), encompassing a transitional region of the lateral cortex whose axons bifurcate and project both through the CC and through the AC. It is likely that one of these branches is eliminated postnatally in normal animals, because these kinds of neurons are no longer seen in later ages. Conceivably, absence of the CC would favor the survival of the AC branch, thereby increasing the number of axons in this commissure.

In the mouse, the midsagittal area of the AC increases rapidly during development until about 6 days after birth (52). Callosal absence is not determined until very late in gestation, about E19 in BALB mice (55), and therefore well within the period of rapid AC growth. Sturrock (51) found that myelination of AC axons starts at about P8–P9 in mice and continues rapidly to about P50. During this time, the size of the AC remains relatively stable; although there is a doubling of axonal diameter due to myelination, which occurs in 12% of axons in the PAC and in 27% of axons in the AAC, there is also a gradual decrease in axon number from P11 to P25 (51). This suggests that the mouse also undergoes an elimination of AC axons during normal development, and it is possible that the number of axons eliminated is reduced when the CC is absent.

The anterior commissure provides interhemispheric transfer of visual, auditory, olfactory and mnemonic information in animals (19) and humans (43, but see 32). Although similar functions are performed by the corpus callosum, cortical areas served by these two commissures have in some cases been found to be distinct (58). The unique use of the anterior commissure as an extracallosal commissural pathway would probably not provide the level of compensation generally seen in acallosals, and it seems likely that the use of several other commissures is also enhanced (35). Subcortical pathways are involved in the transfer of visual information (47).An increased bilateral representation of function in the brain, an increased use of ipsilateral pathways, and the use of behavioral cross-cuing have also been proposed to compensate for absence of the CC (15). No one form of compensation appears to be sufficient to explain the extent of recovery of function displayed in acallosals, and several mechanisms maybe involved. These maybe accompanied by a general reorganization of cortical communication to improve the efficiency of interhemispheric transfer without major rerouting of axons over long distances.

REFERENCES

1. Berbel, P., and G. M. Innocenti. 1988. The development of the corpus callosum in cats: Alight- and electron -microscopic study. *J. Comp. Neurol.* **276:**132–156.
2. Bishop, K. M.,A. Kruyer, and D. Wahlsten. 1996. Agenesis of the corpus callosum and voluntary wheel running in mice. *Psychobiology* **24:**187–194.
3. Cabana, T., and G. F. Martin. 1985. The development of commissural connections of somatic motor-sensory areas of neocortex in the North American opossum. *Anat. Embryol.* **171:**121–128.
4. Chen, Y. P., R. Campbell, J. C. Marshall, and D. W. Zaidel. 1990. Learning a unimanual motor skill by partial commissurotomy patients. *J. Neurol. Neurosurg. Psych.* **53:** 785–788.
5. De Olmos, J. S., and W. R. Ingram. 1972. The projection field of the stria terminalis in the rat brain: An experimental study. *J. Comp. Neurol.* **146:**303–334.
6. Ebner, F. F. 1969. A comparison of primitive forebrain organization in metatherian and eutherian mammals. *Ann. N.Y. Acad. Sci.* **167:**241–257.
7. Elberger, A. J. 1994. Transitory corpus callosum axons projecting throughout developing rat visual cortex revealed by DiI. *Cerebral Cortex* **4:**279–299.
8. Fischer, M., S. B. Ryan, and W. B. Dobyns. 1992. Mechanisms of interhemispheric transfer and patterns of cognitive function in acallosal patients of normal intelligence. *Arch. Neurol.* **49:** 271–277.

9. Granger, E. M., R. B. Masterton, and K. K. Glendenning. 1985. Origin of interhemispheric fibers in acallosal opossum (with a comparison to callosal origins in rat). *J. Comp. Neurol.* **241:** 82–98.
10. Gravel, C., R. Sasseville, and R. Hawkes. 1990. Maturation of the corpus callosum of the rat. II. Influence of thyroid hormones on the number and maturation of axons. *J. Comp. Neurol.* **291:** 147–161.
11. Guadaño-Ferraz, A., F. Escobar del Rey, G. M. Escobar, G. M. Innocenti, and P. Berbel. 1994. The development of the anterior commissure in normal and hypothyroid rats. *Dev. Brain Res.* **81:** 293–308.
12. Hedin-Pereira, C., D. Uziel, and R. Lent. 1992. Bicommissural neurones in the cerebral cortex of developing hamsters. *NeuroReport* **3:** 873–876.
13. Horel, J. A., and D. J. Stelzner. 1981. Neocortical projections of the rat anterior commissure. *Brain Res.* **220:**1–12.
14. Jeeves, M. A. 1991. Stereo perception in callosal agenesis and partial callosotomy. *Neuropsychology* **29:**19–34.
15. Jeeves, M. A. 1992. Compensatory mechanisms—Neural and behavioral: Evidence from prenatal damage to the forebrain commissures. In *Recovery from Brain Damage* (F. D. Rose and D.A. Johnson Eds.),pp. 153–168. Plenum Press,NewYork.
16. Jouandet, M. L., and V. Hartenstein. 1983. Basal telencephalic origins of the anterior commissure of therat. *Exp. Brain Res.* **50:** 183–192.
17. Kadhim, H. J., P. G. Bhide, and D. O. Frost. 1993. Transient axonal branching in the developing corpus callosum. *Cereb. Cort.* **3:**551–566.
18. Kim, J. H. Y., A. Ellman, and J. M. Juraska. 1996. A re-examination of sex differences in axon density and number in the splenium oftherat corpus callosum.*Brain Res.* **740:**47–56.
19. King, C., and W. G. Hall. 1990. Developmental change in unilateral olfactory habituation is mediated by anterior commis-sure maturation. *Behav. Neurosci.* **104:** 796–807.
20. LaMantia, A.-S., and P. Rakic. 1990. Axon overproduction and elimination in the corpus callosum of the developing rhesus monkey. *J. Neurosci.* **10:** 2156–2175.
21. LaMantia, A.-S., and P. Rakic. 1994. Axon overproduction and elimination in the anterior commissure of the developing rhesus monkey. *J. Comp. Neurol.* **340:** 328–336.
22. Lassonde, M., M. Ptito, and F. Lepore. 1990. La plasticitédu systeme calleux. *Rev. Can. Psych.* **44:**166–179.
23. Lassonde, M., H. Sauerwein, A.-J. Chicone, and G. Geoffroy. 1991. Absence of disconnexion syndrome in callosal agenesis and early callosotomy: Brain reorganization or lack of structural specificity during ontogeny? *Neuropsychology* **29:** 481– 495.
24. Lassonde, M., H. Sauerwein, G. Geoffroy, and M. Decarie. 1986. Effects of early and late transection of the corpus callosum in children. *Brain* **109:** 953–967.
25. Lent, R. 1983. Cortico-cortical connections reorganize in hamsters after neonatal transection of the corpus callosum. *Dev. Brain Res.* **11:** 137–142.
26. Lent, R. 1992. Different developmental strategies of the telencephalic commissures: A comparison between the ontogeneses of visual callosal connections and of olfactory commissural connections in rodents. In *The Visual System, from Genesis to Maturity* (R. Lent, Ed.), pp. 131–146. Birkhäuser, Boston.
27. Lent, R., and R. Z. P. Guimara˜es. 1991. Development of paleocortical projections through the anterior commissure of hamsters adopts progressive, not regressive, strategies. *J. Neurobiol.* **22:** 475–498.
28. Lipp, H.-P., and D. Wahlsten. 1992. Absence of the corpus callosum. In *Genetically Defined AnimalModels of Neurobehavioral Dysfunctions* (P. Driscoll, Ed.), pp. 217–252. Birkhäuser, Boston.
29. Livy, D. J. 1996. Interhemispheric axon pathway development in mice with agenesis of the corpus callosum. Ph.D. dissertation, University of Alberta.
30. Livy, D. J., and D. Wahlsten. 1997. Formation of the hippocampal commissure in normal and acallosal mouse embryos. *Hippo-campus* **7:**2–14.
31. Lomber, S. G., B. R. Payne, and A. C. Rosenquist. 1994. The spatial relationship between the cerebral cortex and fiber trajectory through the corpus callosum of the cat. *Behav. Brain Res.* **64:**25–35.
32. McKeever, W. F., K. F. Sullivan, S. M. Ferguson, and M. Rayport. 1981. Typical cerebral hemisphere disconnection deficits following corpus callosum section despite sparing of the anterior commissure. *Neuropsychology* **19:**745–755.

33. Milner, A. D., and M. A. Jeeves. 1979. A review of behavioral studies of agenesis of the corpus callosum. In *Structure and Function of the Cerebral Comm issure* (I. Steele Russell, M. Van Hof, and G. Berlucchi, Eds.), pp. 428–448. MacMillan, New York.
34. Milner, A. D., M. A. Jeeves, P. H. Silver, C. R. Lines, and J. Wilson. 1985. Reaction times to lateralized visual stimuli in callosal agenesis: Stimulus and response factors. *Neuropsychol-ogy* **23:**323–331.
35. Morin, C. L., S. Dolina, R. T. Robertson, and C. E. Ribak. 1994. An inbred epilepsy-prone substrain of BALB/c mice shows absence of the corpus callosum, an abnormal projection to the basal forebrain, and bilateral projections to the thalamus. *Cerebral Cortex* **4:**119–128.
36. Muente, T. F., and H. J. Heinze. 1991. Corpus-Callosum-Agenesie: Interhemisphaerische Integration semantischer Infor-mation. *Nervenarzt* **62:**629–636.
37. Okada, Y., T. Negi, Y. Hori, and T. H. Murakami. 1989. The cortical EEG in the acallosal mouse. *J. Physiol. Soc. Jpn.* **51:** 165–171.
38. Olavarria, J., M. M. Serra-Oller, K. T. Yee, and R. C. van Sluyters. 1988. Topography of interhemispheric connections in neocortex of mice with congenital deficiencies of the callosal commissures. *J. Comp. Neurol.* **270:** 575–590.
39. Ozaki, H. S., T. H. Murakami, T. Toyoshima, and M. Shimada. 1987. The fibers which leave the Probst's longitudinal bundle seen in the brain of an acallosal mouse: A study with the horseradish peroxidase technique. *Brain Res.* **400:**239–246.
40. Persson, G. 1970. Untersuchungen bei drei Fällen mit ange-borenem Balkenmangel. *Psychiat. Neurol. Med. Psychol.* **22:** 448–455.
41. Rauch, R. A., and J. R. Jinkins. 1994. Magnetic resonance imaging of corpus callosum dysgenesis. In *CallosalAgenesis— A Natural Split Brain?* (M. Lassonde and M. A. Jeeves, Eds.), pp. 83–95. Plenum Press, New York.
42. Reeves, A. G. 1991. Behavioral changes following corpus callosotomy. *Adv. Neurol.* **46:** 43 7–443.
43. Risse, G. L., J. LeDoux, S. P. Springer, D. H. Wilson, and M. S. Gazzaniga. 1978. The anterior commissure in man: Functional variation in a multisensory system. *Neuropsychology* **16:**23–3 1.
44. Roy, M. 1996. A test of a genetic model of corpus callosum agenesis and related structures in acallosal mice. B.Sc. Honours thesis, Dept. of Psychology, Univ. of Alberta.
45. Sauerwein, H. C., P. Nolin, and M. Lassonde. 1994. Cognitive functioning in callosal agenesis. In *Callosal Agenesis— A Natural Split Brain?* (M. Lassonde and M. A. Jeeves, Eds.), pp. 221–234. Plenum Press, New York.
46. Schmued, L. C. 1990. A rapid, sensitive histochemical stain for myelin in frozen brain sections. *J. Histochem. Cytochem.* **38:** 717–720.
47. Sergent, J. 1990. Furtive incursions into bicameral minds: Integrative and coordinating role of subcortical structures. *Brain* **109:**537–568.
48. Silver, J., S. E. Lorenz, D. Wahlsten, and J. Coughlin. 1982. Axonal guidance during development of the great cerebral commissures: Descriptive and experimental studies, in vivo, on the role of preformed glial pathways. *J. Comp. Neurol.* **210:** 10–29.
49. Sperry, R. W., M. S. Gazzaniga, and J. E. Bogen. 1969. Interhemispheric relationships: The neocortical commissures; syndromes of hemisphere disconnection. In *Handbook of Clinical Neurology.* Vol. 4. *Disorders of Speech Perception and Symbolic Behavior* (P. J. Vinken and G. W. Bruyn, Eds.), pp. 273–290. Elsevier, New York.
50. Stefanko, S. Z., and V. W. D. Schenk. 1979. Anatomical aspects of the agenesis of the corpus callosum in man. In *Structure and Function of the Cerebral Commissures* (I. Steele Russell, M. Van Hof, and G. Berlucchi, Eds.), pp. 479–482. MacMillan, New York.
51. Sturrock, R. R. 1976. Development of the mouse anterior commissure. I. A comparison of myelination in the anterior and posterior limbs of the anterior commissure of the mouse brain. *Zbl. Vet. Med. C.* **5:** 54–67.
52. Wahlsten, D. 1984. Growth of the mouse corpus callosum. *Dev. Brain Res.* **15:**59–67.
53. Wahlsten, D. 1991. Sample size to detect a planned contrast and a one degree-of-freedom interaction effect. *Psychol. Bull.* **110:** 587–595.
54. Wahlsten, D., and G. Jones. 1983. Structural changes in brains of mice with agenesis of the corpus callosum. *Soc. Neurosci. Abstr.* **9:**494.

55. Wahlsten, D., and H. S. Ozaki. 1994. Defects of the fetal forebrain in acallosal mice. In *Callosal Agenesis—A Natural SplitBrain?*(M. Lassonde and M.A. Jeeves, Eds.),pp. 125–133. Plenum, NewYork.
56. Wahlsten, D., and P. M. Schalomon. 1994. Anew hybrid mouse model for agenesis of the corpus callosum. *Behav. Brain Res.* **64:** 111–117.
57. Wahlsten, D., and V. Sparks. 1995. New recombinant inbred strains expressing 100% total absence of the corpus callosum. *Soc. Neurosci. Abstr.* **21:**796.
58. Zeki, S. M. 1973. Comparison of the cortical degeneration in the visual regions of the temporal lobe of the monkey following section of the anterior commissure and the splenium. *J. Comp. Neurol.* **148:**167–176.